subsequent pages accordingly. Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed

sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By _____
Allen C. Yun
Registration No. 37,971

ACY:pr
624 Ninth Street, N.W.
Washington, D.C. 20001
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
F:\,A\Aoyb\Uemura 8\PTO\notice to comply with seq.wpd

VERSION WITH MARKINGS TO SHOW THE CHANGES MADE

The paragraph beginning at page 9, line 6, has been rewritten as follows:

(5) The protein expression vector according to the above (4), wherein the spacer nucleotide sequence is a nucleotide sequence encoding at least the amino acid sequence of Leu-Val-His-Gly-Lys-Leu (amino acid 24-29 of SEQ ID NO:19);

The second paragraph from the bottom of page 7, has been rewritten as follows:

(8) The protein expression vector according to the above (7), wherein the cleavable nucleotide sequence is a nucleotide sequence encoding at least the amino acid sequence of Asp-Asp-Asp-Asp-Lys (amino acid 19-23 of SEQ ID NO:19);

The paragraph beginning at page 20, line 11, has been rewritten as follows:

For example, a nucleotide sequence encoding an amino acid sequence which is susceptible to enzyme-specific cleavage corresponds to this sequence. Examples thereof include as follows: a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys (amino acid 19-23 of SEQ ID NO:19) (said amino acid sequence is recognized by enterokinase, and the recombinant fusion protein is cleaved at the C-terminus thereof); a nucleotide sequence encoding the amino acid

sequence of Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO:20) (said amino acid sequence is recognized by thrombin, and the recombinant fusion protein is cleaved between Arg-Gly thereof); a nucleotide sequence encoding the amino acid sequence Ile-Glu-Gly-Arg (SEQ ID NO:21) (said amino acid sequence is recognized by factor Xa, and the recombinant fusion protein is cleaved at the C-terminus thereof); a nucleotide sequence encoding the amino acid sequence Glu-Asn-Leu-Tyr-Phe-Gln (SEQ ID NO:22) (said amino acid sequence is recognized by TEV (Tobacco Etch virus) protease, and the recombinant fusion protein is cleaved at the C-terminus thereof), and the like.

The paragraph beginning at page 23, line 6, has been rewritten as follows:

--A space sequence may be, for example, a cleavable sequence from which the secretory signal, the Tag sequence and epitope can be cleaved by enzyme, or the like. In particular, in the case where there is a histidine Tag upstream of the target protein, inserting successively a prepro-region in the secretory signal and inserting the amino acid sequence Leu-Val-His-Gly-Lys-Leu (amino acid 24-29 of SEQ ID NO:19) as a spacer sequence to the C-terminus of the prepro-region are convenient for the cleavage by an enzyme, or the like, because the distance between the trypsin signal and the histidine Tag becomes larger.--

The paragraph beginning at page 25, line 4, has been amended as follows:

The following Examples further illustrate the present invention in detail but are not to be construed to limit the scope of the present invention. In the following Examples, IgGk leader may be understood as a synonym of the secretory signal of IgG. When DDDDK (Asp-Asp-Asp-Asp-Lys) (amino acid 19-23 of SEQ ID NO:19) is present proximate to a trypsin signal, the DDDDK (amino acid 19-23 of SEQ ID NO:19) and the trypsin signal inclusive is called as trypsin signal in some cases (the sequence of 1st to 23rd amino acids in SEQ ID NO: 19), whereas only the trypsin signal without containing said DDDDK (amino acid 19-23 of SEQ ID NO:19) is as called trypsin signal (the sequence of 1st to 18th in SEQ ID NO:19) in other cases. Those skilled in the art can readily understand that a particular sequence corresponds to either of which from the context of the description. The trypsin signal shown in Figs. 1, 3 and 5 refers to the 1st to 18th amino acids in SEQ ID NO: 19. In this connection, IgGk signal and the trypsin signal may be used in an interchangeable manner and, in this ~~resepct~~ respect, both are considered to be equivalent, and the trypsin signal referred to herein may or may not include DDDDK.

The first paragraph beginning at page 31, has been amended as follows:

The portion of pSecTrypHis/Neurosin spanning from the trypsin signal to the enterokinase recognition site was amplified by using SEQ ID NOS: 10 and 11 such that the peptide Leu-Val-His-Gly (amino acid 1-4 of SEQ ID NO;15) was located at the C-terminus. The product was inserted between Nhe I and Hind III sites of pSecTag2A to obtain the plasmid pTrypSig. About 200 bp which contained His tag region in pTrypHis was amplified by using SEQ ID NOS: 11 and 7. A fragment of about 40 bp containing His tag and enterokinase recognition site, which was produced by digesting with Hind III and BamH I, was inserted into pTrypSig to obtain pTrypSigTag (Fig. 5A).

The paragraph beginning at the bottom of page 35, has been amended as follows:

The protein expression vector of the present invention is advantageous and characterized by in that the protein expression vector has the above-described specific construction of the components thereby facilitating the purification and recovery of a target protein in a mature form or an active form. A preferred example of the construction of said protein expression vector includes a secretory signal nucleotide sequence, a Tag nucleotide sequence positioned in the 3' downstream thereof, a cleavable nucleotide sequence comprising a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys (amino acid 36-40 of SEQ ID

NO:19) capable of being recognized by enterokinase, a nucleotide sequence encoding the target protein positioned successively in the downstream, and a nucleotide sequence containing a stop codon positioned in the furthest downstream, where it is possible by using this vector to produce a recombinant protein without additional amino acids attached to the N-terminus or the C-terminus of the target protein, namely the target protein of a mature form or an active form.

IN THE CLAIMS

Claims 5 and 8 have been amended as follows:

5. The protein expression vector according to claim 4, wherein the spacer nucleotide sequence is a nucleotide sequence encoding at least the amino acid sequence of Leu-Val-His-Gly-Lys-Leu (amino acid 24-29 of SEQ ID NO:19).

8. The protein expression vector according to claim 7, wherein the cleavable nucleotide sequence is a nucleotide sequence encoding at least the amino acid sequence of Asp-Asp-Asp-Asp-Lys (amino acid 36-40 of SEQ ID NO:19).